Formation of ectomycorrhizae on *Pinus taeda* seedlings exposed to simulated acidic rain[1]

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Effects of simulated acidic rain on formation of ectomycorrhizae were studied with *Pinus taeda* seedlings grown in plastic trays in a greenhouse. Trays of ectomycorrhizal seedlings were exposed 37 times over 16 weeks to simulated rains adjusted to pH 5.6, 4.0, 3.2, or 2.4. After exposures, mean percentages of short roots that were mycorrhizal (*%M*) were greatest (62.2%) for seedlings exposed to rains of pH 2.4. Values of *%M* exhibited a quadratic relationship with rain acidity (*%M* = 146.49 − 48.96 (rain pH) + 5.68 (rain pH)^2). Values of *R/S* (root/shoot ratio) were negatively correlated ($P < 0.01$) with *%M*. Responses of ectomycorrhiza formation and associated shoot growth to acidity of simulated rains suggest that rains of intermediate acidity (pH 4.0 and 3.2) inhibited ectomycorrhiza formation, or that increased soil acidity or other factors induced by rains at pH 2.4 enhanced ectomycorrhiza formation.

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Les auteurs ont étudié les effets de précipitations acides simulées sur la formation d'ectomycorhizes chez des semis de *Pinus taeda* cultivés en récipients, en serre. Les semis mycorhizés furent exposés à 37 reprises, sur une période de 16 semaines, à des précipitations acides simulées ajustées aux pH 5,6, 4,0, 3,2 ou 2,4. À la fin de la période de traitements, le pourcentage moyen de racines courtes qui étaient mycorhizées (*%M*) fut le plus élevé (62,2%) chez les semis exposés aux précipitations à pH 2,4. Les valeurs de *%M* ont montré l'existence d'une relation quadratique avec l'acidité des précipitations (*%M* = 146,49 − 48,96 (pH de la pluie) + 5,68 (pH de la pluie)^2). Les valeurs de *R/S* (rapport racine/pousse) étaient corrélées négativement ($P < 0,01$) au *%M*. L'effet des précipitations acides simulées sur la formation d'ectomycorhizes et la croissance de la pousse suggèrent que les pluies d'acidité intermédiaire (pH 4,0 et 3,2) inhibent la formation d'ectomycorhizes, ou que l'acidité accrue du sol, ou d'autres facteurs résultant des précipitations à pH 2,4, favorisent la formation d'ectomycorhizes.

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Introduction

Roots of many tree species form ectomycorrhizae. Widespread *Pinus* spp. (all ectomycorrhizal) are distributed on a diverse array of soil types in the southeastern United States. For example, loblolly pine (*Pinus taeda* L.) occurs on many soil types from Texas through Florida and north into Maryland, while other species such as sand pine (*P. clausa* (Englem.) Vasey) are found almost exclusively on sandy soils (11).

Recent investigations indicate that much of the eastern United States and northern Europe currently receive acidic precipitation (14). Approximately 32% of the rainfall samples collected from 1976 to 1979 at three sites in the northeastern United States were characterized by pH < 4 (7). McFee (22) has suggested that many of the soils of the eastern U.S. are potentially sensitive to acid deposition. Ectomycorrhizal forest tree species are distributed over nearly all of these soils.

Effects of simulated acidic rain have been reported for plants (e.g., 7, 12, 38, 40) and soil microorganisms (e.g., 13, 23, 33, 34). Although adverse effects on mycorrhizae have been proposed (35), influences of acidic rain on mycorrhizae have received little attention. Brewer and Heagle (4) found that repeated simulated rains of pH 2.8 applied in addition to ambient rain in the field had no effect on the extent of soybean root colonization by the endomycorrhizal fungus *Glomus geosporum* (Nichol. and Gerd.) Walter. However, sporulation by the fungus within roots of plants exposed to pH 2.8 treatments was only 39% of that observed in plants exposed to pH 5.5 treatments. Haines and Best (8) reported that a single application of solution adjusted with HCl, H_2SO_4, and HNO_3 to pH 3.0 did not visibly damage endomycorrhizae formed by *Glomus mosseae* (Nichol. and Gerd.) Gerd. and Trappe with sweetgum (*Liquidambar styraciflua* L.). Although improved growth of tree seedlings has been reported as an apparent result of ion inputs from simulated acidic rain (27, 40), responses of

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ectomycorrhizae to acidic rain have not been described.

This experiment was conducted to quantify the response of the formation of ectomycorrhizae on loblolly pine seedlings under simulated rain treatments of different acidities.

Materials and methods

Seeds of 10 half-sib families of loblolly pine were mixed in equal quantities by weight, soaked in 30% H_2O_2 for 10 min, and planted in a mixture of peat and vermiculite (1:1, v:v). Seedlings that emerged 13–16 weeks later were transplanted into a mixture of soil (a sandy loam topsoil collected in the North Carolina piedmont and steamed for 2 h at 80°C), peat, and steamed sand (3:1:1 by volume; final composition of the mineral fraction was 79% sand, 15% silt, and 6% clay) in plastic trays (approximately 33 cm long × 13 cm wide × 11 cm deep). Thirty seedlings were transplanted into 2800 mL of the soil mixture in each of the 45 trays. Seedlings were kept in a greenhouse and received tap water daily. Fifteen weeks after the last seedlings had been transplanted, five trays were randomly selected for quantification of incidence of mycorrhizae. The entire mass of roots and soil was removed from each tray, and roots were washed under running tap water. Ten seedlings were randomly selected from each tray. The length of 10 root segments (each 2–7 cm long) selected from each seedling was measured (nearest 0.1 cm), and the numbers of mycorrhizae (bifurcated, coralloid or obviously (at 15×) mantle-covered short roots) and nonmycorrhizal short roots on each segment were counted. Mean root length examined, mean number of ectomycorrhizae, mean number of nonmycorrhizal short roots, and the mean shoot length (nearest 0.1 cm, from the crown to the base of the apical bud) per seedling were calculated for each tray.

The remaining 40 trays of seedlings were exposed to simulated rains in the greenhouse. Simulated rain solutions were prepared with deionized water in four polyethylene tanks. Concentrations of ions characteristic of natural rainfall (6) added were as follows (micrograms per litre): Ca^{2+}, 21.0; K^+, 2.2; Mg^{2+}, 9.8; Na^+, 5.1; NH_4^+, 7.8; Cl^-, 12.0; NO_3^-, 12.0; and SO_4^{2-}, 22.0. Solutions were unamended (pH 5.6 ± 0.2) or were adjusted with a mixture of sulfuric and nitric acids (1*N*; 70 mEq. SO_4^{2-}: 30 mEq. NO_3^-) to pH 4.0, 3.2, or 2.4 (all ±0.1). The pH values of the solutions were determined with a pH meter prior to application. The four solutions were simultaneously pumped at 83 kPa from the tanks through independent delivery systems (constructed of epoxy, neoprene, nylon, polyallomer, polyethylene, polyvinyl chloride, stainless steel, and Tygon® plastic components). Each solution was applied from two stainless steel solid-cone nozzles (Fulljet 1/8 G2.8W, Spraying Systems Co., Wheaton, IL 60187; median volume diameter = 1160 μm (J. Haruch, Spraying Systems Co., personal communication)) suspended 1.2 m above separate greenhouse bench plots. The four solutions were applied to eight bench plots in a randomized complete block design. Five trays of seedlings were placed in each plot (total of 10 trays per acidity level) and were exposed to simulated rain for 30 min. Deposition was estimated in each plot as the mean volume of rain collected in five beakers placed among the trays. Each 30-min application of rain deposited an average of 1.1 cm of solution. Total estimated deposition of H^+, N, and S is presented in Table 1. Immediately after exposure to rain, trays were arranged in a randomized complete block design (10 blocks; one tray from each treatment in each block) on greenhouse benches. Trays of seedlings were returned to the rain simulation plots for an additional 36 exposures over the next 16 weeks.

Plant and soil samples were collected 2 days after the last exposure. The entire mass of roots and soil was removed from each tray and shaken in a polyethylene bucket. Soil from each tray was mixed, and an air-dried soil sample (approximately 350 g) from each tray was analyzed by the North Carolina Department of Agriculture (Agronomic Division, Blue Ridge Road, Raleigh, NC). Shoot length and incidence of ectomycorrhizae on root systems of 10 seedlings from each tray were quantified as described previously. Roots and shoots of 10 additional seedlings from each tray were severed at the root collar, dried separately for 72 h at 105°C, and weighed (nearest 0.01 g). Mean values for length of root examined, number of ectomycorrhizae, number of nonmycorrhizal short roots, shoot length, and shoot and root dry weights per seedling were calculated for each tray. Values for several parameters were anomalous for one tray exposed to simulated rains at pH 2.4, so that tray was rejected from statistical analyses. Results of analyses for soil N were variable and considered unreliable, and they are not presented. Statistical analyses were performed with the mean of each plant-related parameter and the single value for each soil-related parameter from each tray (n = 39).

TABLE 1. Estimated total deposition[a] (milligrams per square metre) of H, N, and S in simulated rain[b]

	Rain acidity (pH)[c]			
Element	5.6	4.0	3.2	2.4
H	1	41	257	1 620
N	113	285	1192	6 917
S	143	602	3023	18 287

[a]Based on ion concentrations in rain solutions and the amount of simulated rain deposited in plots.
[b]37 simulated rains (each 1.1 cm) were applied over 16 weeks.
[c]Rain acidity adjusted with H_2SO_4 + HNO_3.

Results

The mean shoot length of seedlings in five randomly selected trays at the beginning of this experiment was 14.9 cm. A mean of 299.0 cm of roots from 10 seedlings was examined from each tray. A mean of 3.8 short roots (ectomycorrhizae plus nonmycorrhizal short roots) per centimetre of root occurred, and 39.6% of these were visibly ectomycorrhizal.

Values of the mean percentage of short roots that were rated as "ectomycorrhizae" (*%M*) per seedling in each tray at the end of this experiment ranged from 30.7 to 70.4%. The General Linear Model procedure (GLM) (1) indicated no significant differences ($P < 0.05$ required) in values of *%M* (the parameter of primary interest) among seedlings kept on two separate benches and exposed in separate blocks of the rain simulator throughout the experiment. Subsequently, all data were subjected to GLM as a randomized complete block design with 10 replications each for three acidity levels (pH 5.6, 4.0, and 3.2) and 9 replications for the fourth (pH 2.4). Overall responses of selected parameters to rain acidity were defined with least squares regression. *F*-tests for comparisons of selected means were obtained with the "CONTRAST" option of GLM (1).

No foliar injury was visible on seedlings exposed 37 times over 16 weeks to rains at pH 5.6, 4.0, or 3.2. Approximately 20% of the needle area of seedlings exposed to rains at pH 2.4 was necrotic. Shoot length did not vary significantly among treatments (mean = 15.5 cm). However, needles on seedlings exposed to rains at pH 2.4 were longer, and nonnecrotic portions were darker green than needles on seedlings exposed to other solutions. Shoot dry weights were greatest for seedlings exposed to rains at pH 2.4.

A mean of 291.6 cm of roots from 10 seedlings from each of the 39 trays was examined for ectomycorrhizae. Data relating *%M* and rain acidity were fit by a quadratic model ($\%M = 146.49 - 48.96$ (rain pH) $+ 5.68$ (rain pH)^2; $F = 13.92$ ($P < 0.01$), $r = 0.66$). Mean *%M* was greatest (62.2%) for seedlings exposed to rains at pH 2.4 and least (42.5%) for seedlings exposed to rains at pH 4.0. Mean *%M* for seedlings exposed to rains at pH 4.0 was significantly less than that for

TABLE 2. Characteristics of loblolly pine seedlings[a] after exposures to simulated rains[b]

Seedling characteristic[c]	Rain acidity (pH)[c]				F[d]			
	5.6	4.0	3.2	2.4	All four	5.6 vs. 4.0	2.4 vs. all others	5.6 and 2.4 vs. 4.0 and 3.2
%M[f]	50.4	42.5	46.8	62.2	9.80**	4.54*	23.04**	18.51**
Short roots per centimetre of root	4.2	4.1	3.8	3.6	2.22	—[g]	—	—
Mycorrhizae per centimetre of root	2.1	1.7	1.8	2.2	3.98*	4.52*	6.84*	11.69**
Root dry weight (g)	0.29	0.30	0.23	0.25	1.84	—	—	—
Shoot dry weight (g)	0.64	0.58	0.52	0.72	2.78[h]	0.55	4.43*	5.68*
R/S[i]	0.45	0.53	0.45	0.36	5.97**	3.51	12.64**	8.83**

[a]30 seedlings per tray.
[b]37 simulated rains (each 1.1 cm) were applied over 16 weeks.
[c]Rain acidity was adjusted with H_2SO_4 + HNO_3.
[d]*F*-tests for variation among means for an unbalanced design (because of one missing value for each seedling characteristic determined for trays exposed to simulated rains at pH 2.4) analyzed by the General Linear Model procedure of the Statistical Analysis System (see reference 1). Values followed by * or ** denote significant differences among the specified means at $P < 0.05$ or $P < 0.01$, respectively.
[e]Values tabulated for each characteristic are means for 10 trays of seedlings per acidity level (one value per tray, determined as the mean for 10 seedlings per tray) for pH 5.6, 4.0, or 3.2 , and the mean for nine trays for pH 2.4.
[f]Percent of short roots that were ectomycorrhizae.
[g]*F* value for specific contrast not calculated because *F* value for all four means indicates no significant differences among them.
[h]Significant at $P = 0.06$.
[i]Ratio of root/shoot dry weight.

seedlings exposed to rains at pH 5.6 (Table 2). A trend of fewer short roots (mycorrhizae plus nonmycorrhizal short roots) per centimetre of root associated with greater rain acidities was not significant. However, the number of mycorrhizae per centimetre of root varied with rain acidity in a manner similar to that exhibited by values of *%M* (Table 2), and the frequency of mycorrhizae along roots was significantly correlated with *%M* ($r = 0.79$, $P < 0.01$).

Data relating values of the mean root/shoot ratio (*R/S*, calculated for each tray with mean root and shoot dry weights) and rain acidity were also fit by a quadratic model ($R/S = -0.3315 + 0.3918$ (rain pH) $- 0.0449$ (rain pH)^2; $F = 9.21$ ($P < 0.01$), $r = 0.58$). Mean *R/S* was least (0.36) for seedlings exposed to rains at pH 2.4 and greatest (0.53) for seedlings exposed to rains at pH 4.0. No significant variation in root dry weights occurred with respect to rain acidity, so the small *R/S* values associated with rains at pH 2.4 reflect greater dry weights of shoots associated with that treatment (Table 2). Values of *%M* were correlated directly with shoot dry weights ($r = 0.60$, $P < 0.01$) and negatively with *R/S* ($r = -0.52$, $P < 0.01$).

Results of soil analyses conducted at the end of the experiment indicated significant variation in several soil properties with respect to rain acidity (Table 3). Soil pH values from trays exposed to rains at pH 5.6, 4.0, or 3.2 ranged from pH 5.69 to 7.10. Values in trays exposed to rains at pH 2.4 ranged from pH 4.75 to 5.36. The relationship between values of *%M* and values of soil pH was fit by a linear model (*%M* = 113.19 − 10.18 (soil pH); $F = 29.54$ ($P < 0.01$), $r = 0.67$).

Discussion

Infection of seedlings in the greenhouse in this experiment occurred from ambient inocula in the air in the North Carolina piedmont. The ectomycorrhizal status of seedlings immediately prior to initiation of rain treatments demonstrates the reliability of mycorrhizal formation under such circumstances. Indeed, elaborate precautions must be taken to prevent infection (18).

Chemical characteristics of the soil mix were not determined before exposures to rain were initiated. Values of *%M* and *R/S* must be interpreted with reference to acidity levels in simulated rains and results of soil chemical analyses conducted at the end of the experiment. Although an initial soil acidity of pH 6.5−7.0 and an initial cation exchange capacity of 4−5 mEq./100 mL are suggested by data for soil mix exposed to rains at pH 5.6 or 4.0 (Table 3), results cannot be reliably expressed in terms of changes in soil properties that may have been induced by different levels of acid deposition.

Chemical analyses after exposures (Table 3) indicate that characteristics of soil exposed to rains at pH 4.0 were very similar to those of soil exposed to rains at pH 5.6. Only concentrations of Zn varied significantly between the two treatment acidities, but the small change is of doubtful biological significance. Because concentrations of major basic cations did not vary significantly, the apparent increase of cation exchange capacity of soils exposed to rains at pH 3.2 or 2.4 is probably related to retention of H^+ (as exchangeable acidity) and SO_4^{2-}.

Rains at pH 4.0 or 3.2 were either inhibitory or less stimulatory to ectomycorrhiza formation compared with rains at pH 5.6. Values of *%M* for seedlings exposed to rains at pH 4.0 were significantly less than those for seedlings exposed to rains at pH 5.6. Substances deposited in rains may have interacted with soil factors or affected plant metabolism and resulted in inhibition of formation of ectomycorrhizae. Antifungal effects of sulfur (15) or changes in root exudates and rhizosphere microbial populations could have inhibited formation of ectomycorrhizae. Differences in the mycorrhizal status of seedlings exposed to simulated rains at pH 5.6 or 4.0 despite the apparent lack of changes in soil chemical characteristics suggest indirect modes of action of rain chemistry on mycorrhizae. Qualitative changes in rhizosphere populations possibly owing to alterations in root exudates have been observed in association with simulated rains at pH 3.5 (23). Nodulation of kidney bean (*Phaseolus vulgaris* L.) by *Rhizobium phaseoli* Dangeard was inhibited more by simulated rains at pH 3.2 onto plants and soil than by drenches onto soil alone with solution at the same acidity (compared with rain or drench treatments with pH 5.7

TABLE 3. Properties[a] of soil mix[b] from trays of pine seedlings after exposure to simulated rains[c]

Soil property	Rain acidity (pH)[d]				F[e]			
	5.6	4.0	3.2	2.4	All four	5.6 vs. 4.0	2.4 vs. all others	5.6 and 2.4 vs. 4.0 and 3.2
pH[f]	6.49	6.73	6.41	5.02	68.22**	3.55	189.29**	77.77**
Ac[g] (mEq./100 mL)	0.44	0.34	0.78	1.82	37.84**	0.45	100.78**	27.88**
K[h,i] (mEq./100 mL)	0.05	0.04	0.04	0.04	2.79	—[j]	—	—
Ca[h,k] (mEq./100 mL)	2.23	2.15	2.23	2.41	0.96	—	—	—
Mg[h,k] (mEq./100 mL)	1.73	1.57	1.67	1.90	1.43	—	—	—
Na[h,i] (mEq./100 mL)	0.19	0.12	0.12	0.16	1.63	—	—	—
CEC[l] (mEq./100 mL)	4.65	4.22	4.84	6.32	8.90**	1.05	22.84**	9.75**
BS[m]	90.8	93.1	84.6	70.9	30.76**	0.87	77.77**	20.52**
Mn[h,k] (mg/1000 mL)	11.8	11.6	11.9	10.7	1.99	—	—	—
Zn[h,k] (mg/1000 mL)	5.2	3.9	4.1	5.5	4.29*	5.54*	5.39*	11.79**
Cu[h,k] (mg/1000 mL)	0.5	0.5	0.4	0.5	4.99**	0.18	12.06**	9.35**
P[h,n] (mg/1000 mL)	40	41	38	35	1.57	—	—	—
S[o] (mg/1000 mL)	22.8	8.3	58.5	350.5	133.61**	0.58	384.09**	125.38**

[a]Values are means of soil test values for 10 trays per acidity level (one sample per tray) for pH 5.6, 4.0, or 3.2, and the mean for nine trays for pH 2.4.

[b]3:1:1 soil−peat−sand (by volume).

[c]37 simulated rains (each 1.1 cm) were applied over 16 weeks.

[d]Rain acidity was adjusted with H_2SO_4 + HNO_3.

[e]F-tests for variation among means for an unbalanced design (because of one missing value for each soil property determined for soil mix in trays exposed to simulated rains at pH 2.4) analyzed by the General Linear Model procedure of the Statistical Analysis System (see reference 1). Values followed by * or ** denote significant differences among the specified means at $P < 0.05$ or $P < 0.01$, respectively.

[f]Determined by suspension of 1:1 soil−water (by volume).

[g]Exchangeable acidity, in pH 6.6 buffer.

[h]Determined after extraction with Mehlich 3 extractant (see reference 24).

[i]Determined by flame photometry.

[j]F value for specific contrast not calculated because F value for all means indicates no significant variation among them.

[k]Determined by atomic absorption spectrophotometry.

[l]Cation exchange capacity, in pH 6.6 buffer.

[m]Percent base saturation, ((K + Ca + Mg + Na)/CEC) × 100%.

[n]Determined colorimetrically (molybdenum blue).

[o]S in SO_4^{2-} form, extracted with NH_4Cl, precipitated as $BaSO_4$, and determined turbidimetrically.

solution) (38). In another experiment (31), applications of simulated rains (pH 3.2 vs. 6.0) to kidney bean foliage inhibited nodulation more than applications to soil only or to soil and foliage. A plant-mediated effect of rain acidity on nodulation was suggested in both reports (31, 38). Inhibition of reproduction by root-knot nematodes (*Meloidogyne hapla* Chitwood) may have also been due to effects of simulated rains at pH 3.2 on the physiology of the host plants (30). Similar indirect effects on ectomycorrhiza−plant−soil relations may have occurred in the present study in association with rains at pH 4.0 and 3.2.

Significant correlations among shoot dry weights, *%M*, and *R/S* values are mutually supportive. Enhanced shoot growth, and hence lower *R/S* ratios, are commonly reported responses to the benefits of improved mycorrhiza formation (5, 29; studies reviewed in 9). In the present experiment the observed quadratic relationship of *R/S* values with rain acidity is generally predictable based on the relationship between *%M* values and rain acidity.

Both statistical examinations of data relating ectomycorrhiza formation and rain acidity (the quadratic equation that defines the dose−response relationship between values of rain pH and *%M*, and *F*-tests for contrasts of treatment means) demonstrate that formation of ectomycorrhizae was less pronounced when seedlings were exposed to rains at pH 4.0 than at pH 5.6. However, data relating shoot dry weights and rain acidity were not fit by a quadratic model, and an *F*-test suggested that shoot dry weights for seedlings exposed to rains at pH 5.6 or 4.0 were not significantly different. Although data relating *R/S* and rain acidity were fit by a quadratic model, the *F*-test for means associated with rains at pH 5.6 vs. 4.0 suggested that variation in *R/S* between these two acidity levels alone was not significant. Hence, no striking differences occurred in growth response of seedlings to the difference in ectomycorrhiza formation associated with the two least-acidic treatment regimes. The magnitude of the difference in *%M* (16%) between seedlings exposed to rains at pH 5.6 or 4.0 may have been insufficient to induce a major difference in seedling growth over the short duration of the experiment.

Conditions responsible for lower values of *%M* associated with rains of intermediate acidity may have been offset by a stimulatory effect of rains at pH 2.4. Accumulation in soil of H^+ may have contributed to the enhancement of ectomycorrhiza formation on seedlings exposed to the most acidic rains. Ectomycorrhizal fungi are generally acidophilic (24, 30, 36) and several studies have demonstrated enhanced formation of ectomycorrhizae with increased substrate acidity (21, 25, 26, 36). In the present experiment, values of *%M* exhibited a linear relationship to soil pH values determined at the end of the experiment. Soil from all trays exposed to rains at pH 2.4 had lower pH values than soil from all other treatments, and the mean *%M* value was greatest for these trays. Thus, growth of ectomycorrhizal fungi promoted by increased soil acidity may have contributed to improved ectomycorrhiza formation. The two conflicting hypothetical effects (mycorrhizal suppression via indirect mechanisms at intermediate acidity vs. stimulation of mycorrhizal fungi by soil acidity associated with rains at pH 2.4) would explain the quadratic relationship between values of *%M* and rain pH. The relationship between acid deposition and ectomycorrhiza formation remains uncertain for

soil exposed to rains at pH 2.4 and with a final pH < 4.75. Further acidification of the soil associated with long-term acid deposition could eventually reverse the stimulatory trend.

Effects of acid deposition on ectomycorrhizae may vary with the characteristics of the plant–fungus–soil system. Occasional sporocarps of *Thelephora terrestris* (Ehrh.) Fr. and *Laccaria laccata* (Scop. ex Fr.) Berk. and Br. occurred in trays, and at least 95% of all ectomycorrhizae had characteristics of infection by these two fungi (19, 20). Infection of pine seedlings by *T. terrestris* in soil previously uncolonized by ectomycorrhizal fungi is common (19), but the species composition of fungi that form ectomycorrhizae with a given plant often changes as the plant ages (3, 19, 39). Different fungi may form ectomycorrhizae on different roots of the same tree when roots have colonized different substrates (10, 37). Thus, interactions of roots of mature trees with other ectomycorrhizal fungi and acid deposition may differ from those described here. Different fungi also vary in adaptability to acidic conditions, such as spoil banks from coal strip mines (2, 16, 17, 28). Although no variations in frequency of morphological types of ectomycorrhizae with respect to rain acidity were noted in the present experiment, shifts in fungal species that form ectomycorrhizae as soil acidity changes have been suggested (32, 36). Hence, changes in chemical and biological characteristics of soil exposed to acid deposition over years may simply induce shifts in species of ectomycorrhizal fungi that occur with roots, rather than alter the overall incidence of mycorrhizae. Effects observed associated with specific levels of acidity in precipitation may also vary with chemical characteristics of soils at different sites.

Conclusions from this experiment must be drawn with caution owing to the relatively short duration and greenhouse conditions of the study. Leaching and ion accumulation characteristics of the soil mixture in trays differed from those of a forest soil. However, growth response data strongly support observations relating formation of ectomycorrhizae and rain acidity. Results of this study suggest that rainfall of intermediate acidity can inhibit formation of ectomycorrhizae. However, continued rainfall of extreme acidity may enhance formation of ectomycorrhizae, perhaps partially through inducement of an increasingly acidic soil environment or other alterations of soil chemistry. Stimulation of ectomycorrhiza formation by soil chemistry modification might be particularly pronounced with certain *Pinus* spp. and other ectomycorrhizal forest tree species that occur on poorly buffered soils that regularly receive acid deposition. Because some fungi seem well adapted to adverse conditions (16, 17), the natural occurrence of various fungi may further modify ectomycorrhizal response to acid deposition. Additional studies are required for verification of these hypotheses and improvement of the description of the dose–response relationship between acid deposition and formation of ectomycorrhizae. Furthermore, accurate prediction of the impact on long-term forest productivity by these phenomena, especially when considered with the degree of foliar necrosis and nutrient leaching losses potentially induced by acidic rain, will require detailed investigation.

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